

The University of Chicago

A QUINHYDRONE-DILUTION
METHOD FOR THE pH
OF BLOOD SERUM

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1933

By
PAUL BLAINE DONOVAN

Private Edition, Distributed by
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ACKNOWLEDGMENT

The author wishes to express his grateful appreciation of the assistance he received from Dr. Martin E. Hanke, who helped with both the experimental work and the writing of this dissertation, and from Miss Martha Johnson and Mr. R. B. Oesting, who made most of the gasometric analyses.



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INTRODUCTION

The quinhydrone electrode is easily constructed, easily manipulated, and remarkably quick in attaining equilibrium; it is, in short, a most convenient tool for determining hydrogen ions. Its usefulness in many fields of investigation has been thoroughly established, but it has not been readily applicable to a study of blood and serum.

The reports of its use with blood are contradictory. Corran and Lewis (1), for example, report very good results with whole blood, and Mislowitzer (2) reports favorable comparisons of the quinhydrone and hydrogen electrode pH values of slightly diluted blood; but Cullen and Billmann, Mozolowski, Vellinger and Roche, Runge and Schmidt and many other investigators think the quinhydrone electrode unsuitable for determining the pH of blood (3, 4, 5, 6). The chief difficulty seems to be a change of E. M. F. with time--a drift in potential--so rapid as to preclude obtaining values that are certain within 5 or 6 millivolts. Variations of this order would produce variations of about 0.1 pH.

The drift in the case of serum is not so rapid, and a number of workers report that they obtain E.M.F. values--usually by taking several successive readings and extrapolating the values to zero time--from which they calculate pH numbers that differ from those obtained by other methods by a more or less constant difference. Cullen and Earle, for example, added quinhydrone to undiluted serum, extrapolated to zero time and ob-

tained E.M.F. values which seldom varied more than 0.5 or 0.6 millivolt. From these values, they calculated pH numbers that averaged 0.06 pH more acid than the hydrogen electrode pH with human serum and 0.09 pH more acid with dog serum (7). Mislowitzer, on the other hand, diluted serum and plasma with a 0.9% NaCl solution containing quinhydrone, and, using the fifteen second E.M.F. value, calculated pH numbers that were 0 to 0.05 pH more alkaline than the corresponding hydrogen electrode pH values (2).

Recently, Dr. Martin E. Hanke, of this laboratory, observed that the addition of a sufficiently small amount of quinhydrone to serum causes a drift toward more negative potentials, whereas relatively large amounts cause a positive drift. Further study showed that there is an optimum concentration of quinhydrone which practically eliminates the drift. Since this concentration would be difficult to control by adding solid quinhydrone to serum, Dr. Hanke devised a method whereby it can be readily controlled by mixing measured volumes of serum and quinhydrone solution in graduated capillary electrode vessels (8). Since the elimination of the drift depends on the concentration of quinhydrone in the mixture, and since the direction and rate of the drift show the nature and extent of the divergence from the optimum concentration, the proper relative volume of quinhydrone solution needed to eliminate the drift is quickly ascertained. This proper relative volume of quinhydrone solution will be called the proper or effective dilution. Dr. Hanke found that serum can be effectively diluted with either water, or 0.9% or 10% NaCl solutions, saturated with quinhydrone, but that the reproducibility of E.M.F. is better with the salt solutions (9). That is, successive determinations at the effective dilution showed agreement among themselves of 0.2 to 0.5 millivolts with the salt

solutions, but they might vary by a millivolt or more with the water solutions.

These studies made a long stride toward an accurate quinhydrone method for the pH of serum in that they showed that the tendencies to drift toward more negative and more positive potentials can be balanced repeatedly at very nearly the same E.M.F. value. They did not show whether or not the peculiar behavior of the quinhydrone electrode in serum is due to any known mechanism--for example, the salt and protein errors shown by Linderström-Lang (10, 11) and others to be inherent in the method--but the answer is not essential to the development of an accurate quinhydrone method. This last could be accomplished only by exploring a wide variety of quinhydrone procedures, comparing them with a standard method and selecting the procedure which best matched the standard method; for the quinhydrone method is an empirical method in which potentials are observed and pH values are so calculated from them, by the use of empirical constants and corrections, that they will match those of a standard method. The studies already made pointed the way for further studies, and it was decided to continue them as the subject for a thesis.

A series of collaborative experiments being conducted by Dr. Hanke afforded an excellent opportunity to explore a large number of quinhydrone procedures and to compare them with the gasometric method being used in his experiments. A large number of solutions, varying in the kind of salt used and in the concentrations of both salt and quinhydrone, have been studied and a procedure has been selected which checks the gasometric method within its experimental error. This procedure involves the effective dilution of 1 volume of serum with about 1.8 volumes

of a solution containing 10 grams of KCl and 125 milligrams of quinhydrone per 100 cc. of solution, and will be described in detail later. The following pages recount the development of this procedure and show the reasons for its selection.

APPARATUS AND EXPERIMENTAL PROCEDURES

A. Drawing of Blood and Preparation And Storing of Serum

Dog's blood, taken from dogs anaesthetized with sodium barbital, was used exclusively, but the manner of drawing and centrifuging the blood and separating the serum depended on whether or not the serum was to be equilibrated. Blood for equilibrated samples was simply allowed to flow from a carotid artery into open centrifuge tubes and centrifuged at room temperature. The clear serum was promptly decanted or pipetted, with a minimum of cells, into a small Erlenmeyer flask and stored in a refrigerator until needed. Blood for the non-equilibrated samples, on the other hand, was drawn and centrifuged without exposure to air by means of a special type of centrifuge tube and technique recently described by Dr. Hanke (12). The centrifuging of the blood and the transfer of the serum to serum tonometers took place in a room kept at 38°C., and care was taken to avoid exposure to air throughout the whole process. Once in the serum tonometers, the serum samples were promptly removed to a room kept at 23°C. and were analyzed within 10 hours by the quinhydrone method.

B. The Gasometric pH Determination

Equilibrated samples of serum were used almost exclusively in the quinhydrone electrode studies, and it was our practice to prepare one or two large samples and several small ones. The pH values of all the samples were determined by both the quinhydrone and gasometric methods, and the remainder of the large samples was used in a more detailed study of various quinhydrone procedures.

1. Equilibration

As soon as convenient, the serum for the equilibrated samples was transferred, in 10 to 50 cc. portions, to 300 cc. gas tonometers which contained various mixtures of CO_2 and air. These gas tonometers were then slowly rotated in a room kept at $38^\circ\text{C}.$, and their stop-cocks were opened momentarily, at intervals of 5 to 15 minutes, to make the pressures within the tonometers exactly atmospheric. After about 30 minutes, when the temperature of the serum was the same as that of the room, especial care was taken to keep the latter within 0.1° of $38^\circ\text{C}.$, and, when at this temperature opening the stop-cocks caused no movement of gas, the barometric pressure was noted. About ten minutes later, the rotator was stopped, the serum promptly transferred without exposure to air to serum tonometers, and the samples set in the $23^\circ\text{C}.$ room to cool. The gas tonometers were fitted at once with mercury leveling bulbs and the gas was kept under a pressure slightly greater than atmospheric until analyzed for CO_2 .

2. Analysis

The gasometric analyses involved obtaining experimental data and calculating pH numbers from these data by the use of certain empirical constants. The experimental data included the serum CO₂ content and the percent CO₂ in the gas mixtures and were obtained by the procedures described by Van Slyke and his co-workers (13, 14). The pH was calculated by substituting the values for the serum CO₂ content, [CO₂], and the partial pressure of CO₂ in the gas mixture, pCO₂,--calculated from the percent CO₂ in the gas mixture and the barometric pressure--in the Henderson Hasselbalch equation:

$$\text{pH} = \text{pK}' + \log \frac{[\text{CO}_2] - K_0 \times \text{pCO}_2}{K_0 \times \text{pCO}_2} .$$

The constants used in the above calculation, namely pK' = 6.10 and K₀ = 0.0301, are also the ones given by Van Slyke (15).

3. Accuracy of the Gasometric Method

The accuracy of the gasometric method just described has been discussed recently by Van Slyke, Sendroy and Liu, who claim that its maximum error was 0.02 pH when used with a miscellaneous lot of sera (16). They allocate the errors to various sources as follows:

Due to variations in pK'	0.01 pH
Due to variations in α (used in calculating K ₀)	.004pH
Due to errors of 1% in determining pCO ₂	.004pH
Due to errors of 0.1 mM in determining CO ₂	.002pH

The sum of the above errors is the maximum error to be allowed for in evaluating the absolute pH of a sample of serum, but differences among samples of the same serum, equilibrated with various mixtures of CO₂ and air, can be determined with a maximum

error of 0.006 pH, since the variations in pK' and λ are avoided.

The statement was previously made that the selected quinhydrone-dilution method checked the gasometric method within the experimental error of the latter. That statement can now be amplified to show more exactly the agreement between the two methods. In more than 60 samples of serum, ranging in pH from 7.0 to 7.7, the pH values determined by the chosen quinhydrone procedure checked the gasometric pH values with an average deviation of about 0.006 pH and a maximum deviation of 0.016 pH. Moreover, the differences in pH among equilibrated samples of the same serum, ranging in pH from 7.3 to 7.5, as determined by the quinhydrone method, checked those determined by the gasometric method with a maximum error of about 0.006 pH.

C. The Quinhydrone pH Determinations

1. Precautions

The constitution of a sample of serum may be sufficiently altered by slight exposure to air, or by standing too long, to make an appreciable difference in the pH as determined by the quinhydrone method. Precautions must be taken, therefore, to avoid these sources of error.

Since the care taken to avoid exposure to air at all the critical stages until the serum is safely stored in the serum tonometers has been mentioned, only the precautions taken in sampling the serum for the quinhydrone determinations will be described here. These precautions were made possible by a type of serum tonometer especially adapted for sampling serum with the graduated capillary electrode vessel. The tonometer is

equipped with a stop-cock and mercury leveling bulb, as usual, but has the capillary tube projecting beyond the stop-cock drawn out to form a cup of 2 or 3 cc. capacity. This cup was kept partly full of mercury, thus sealing the serum in the capillary from the air, and the serum in the electrode vessel was sealed from the air, after sampling, by filling the tip of the electrode vessel with mercury before withdrawing it from the cup.

The length of time that serum can stand before the pH by the quinhydrone method is appreciably altered depends on the temperature. A sample of serum may be apparently stable at 38°C. for as much as half an hour and then decrease 0.02 or 0.03 pH in the next half-hour, the rate of change increasing with the time. The same sample would have been stable for at least 10 or 12 hours at 25°C. and it might show less than 0.01 pH change after several days in the refrigerator if it had been put there within an hour or so after being transferred to the serum tonometer. Because of these facts we found it much more accurate as well as convenient to determine the quinhydrone pH values at 20° to 25°C. and then apply an empirical correction to convert them to the pH values at 38°C., than to determine the quinhydrone E.M.F.'s directly at 38°C.

2. Apparatus

The quinhydrone determinations were made with a modification of the conventional set-up. We used a Leeds and Northrup Type K potentiometer and the Leeds and Northrup galvanometer adapted to it, but we used a new type of quinhydrone half-cell and a modification of the usual type of salt bridge connecting the quinhydrone and calomel half-cells.

a. The electrode vessel.-- The electrode vessel

devised by Dr. Hanke resembles the Cullen-Biilmann micro-vessel except that the outer tube is graduated. It consists of two glass tubes of narrow bore that fit snugly one inside the other. One end of each tube is slightly tapered, and they are held together by a short piece of narrow bore rubber tubing which is stretched over the untapered end of the outer tube and holds the inner tube with an air-tight grip. The outer tube, which has a bore of about 3 mm., has etched marks dividing about 0.6 cc. of its volume into 0.01 cc. units, so that measured volumes of liquid can be drawn into or expelled from it by moving the inner tube in relation to these marks. A platinum wire, 0.5-0.75 mm. in diameter, is sealed in the tapered end of the inner tube, with about two centimeters of its length protruding beyond the seal, so that it is bathed in the liquid drawn into the outer tube. The sensitivity of the electrode is a function of the area of the platinum wire, and wires thinner than 0.5 mm. in diameter are not sufficiently sensitive for our purpose. The platinum wire is fused in turn to a copper wire which extends beyond the open end of the inner tube and can be connected with the potentiometer. In order to be able to make this connection with maximum speed, a spring clip was fixed to the wire leading from the potentiometer, which could be clipped on to the copper wire of the electrode in about one second. The vessel is charged by drawing in an excess of liquid, inverting the vessel and tapping it gently with the finger-tips to remove air bubbles and expelling all but the desired volume of liquid. If a second liquid is to be added to the first, its volume is measured as it is drawn into the vessel.

b. The liquid junction.-- The liquid junction consists of a large beaker of saturated KCl solution which is connected with the saturated calomel half-cell by means of an agar-KCl bridge. The agar-KCl bridge is put in place before beginning a series of determinations, and the calomel half-cell is connected with the potentiometer, so that when, after being charged, the electrode vessel is clamped upright in the beaker of saturated KCl solution, nothing remains to be done except to connect the electrode with the spring clip of the potentiometer wire and take the reading. The beaker of KCl solution and the calomel half-cell are both covered with mineral oil to prevent evaporation and creeping of the salt, and the whole set-up, except the electrode vessel, usually remains fixed during a whole series of determinations. This adds to the speed and accuracy of the determinations; for one needs to make only one connection and avoids the possible variations introduced by changing salt bridges.

The beaker of saturated KCl solution served also as a constant temperature bath for the quinhydrone half-cell and was equipped with a standardized thermometer, graduated to tenths of a degree, so that its temperature could be accurately known. The saturated calomel half-cell was also equipped with a standardized thermometer and the temperature of both the calomel and quinhydrone half-cells was recorded for every determination. Generally, since the determinations were made in a room the temperature of which seldom varied by more than a few tenths of a degree, the temperatures recorded did not vary more than 0.2° or 0.3°C. during a whole series of determinations; but if either did, the E.M.F. of the phosphate standard was read more frequently and the E.M.F.'s of the samples of serum were corrected to the

temperature of the standard nearest their own. The temperature corrections, which will be discussed in detail later, were thus kept from exceeding 0.6 millivolt.

3. Standards

Two standards were used: (1) an N/10 HCl solution, made up from the constant boiling acid and conductivity water, which was assigned the pH number of 1.08 and used as ultimate standard, and (2) a phosphate buffer, having a pH near that of the serum, which was used as a working standard. The phosphate mixture had a pH of about 7.2, since that mixture gives better duplicability than one having a pH of 7.4, and was made up according to the directions of Cullen. Its pH was checked against that of the ultimate standard for every series of determinations (17).

We had difficulty in reading the potentials of the N/10 HCl solution when it was immersed in the saturated KCl bath, and the pH values of our phosphate mixtures standardized with it did not agree well with those given by Hastings and Sendroy as the hydrogen electrode pH values of the same mixtures (18). The potential of the N/10 HCl drifts--usually toward more negative values--in the saturated KCl bath at the rate of 0.3 or 0.4 mv. in the first minute or two and then slows down. If, after the first more rapid drift has slowed down, some of the liquid be expelled from the electrode vessel, the E.M.F. reverts to a higher value and begins drifting again. In our earlier experiments, we used this highest E.M.F. value in calculating the pH values of our phosphate standards, but even so these values were 0.01 to 0.02 pH more acid than those of Hastings and Sendroy. We later observed that the addition of HCl to the bath tends to

eliminate the drift and to increase the E.M.F. values. Further investigation showed that the drift is practically eliminated when the bath is 0.02 N with respect to HCl and that the pH values of the phosphate standards calculated from the increased E.M.F. values of the N/10 HCl agree very well with those of Hastings and Sendroy. The potentials of the phosphate buffers, however, drift in the KCl-HCl bath, so we continued to use the saturated KCl bath without HCl for the phosphate standard and the serum.

4. Procedure with a standard

The same electrode vessel was used for both serum and standards, and its reliability was checked by reading the E.M.F.'s of the standards with one or two other vessels as well. The standard solutions were used for this purpose because of the ease of obtaining duplicate E.M.F. readings, and because no care need be taken to measure the volume drawn into the electrode vessel. An excess of solid quinhydrone, about 20-100 mgms., is simply added to 3 or 4 cc. of the standard solution in a small vial, the mixture stirred about one-half minute to insure solution of the quinhydrone, and after 2 or 3 rinsings with the solution, the electrode vessel is filled with it. Air bubbles are removed and all but 0.3 or 0.4 cc. of the solution expelled. The connections are then made as described and the potential is read.

5. Procedure with serum

The procedure in the case of serum is a little more

complicated than with the standards. The electrode vessel is taken apart and thoroughly rinsed, first with distilled water and then with a 10% KCl solution containing 125 mgms. of quinhydrone per 100 cc. of solution. It is then filled with the quinhydrone-salt solution, the air bubbles removed and the solution expelled to the 0.25 cc. mark. A small globule of mercury--about 0.05 cc.--is next drawn into the vessel and this is followed by 0.15 cc. of serum. The serum is sealed from the air with another globule of mercury, a finger is placed over the tip of the vessel, the time is noted, and the vessel is shaken and inverted for 15 or 20 seconds. Next, all the mercury and a drop or two of the diluted serum are expelled, and the vessel is clamped upright in the KCl bath. The spring clip of the potentiometer wire is attached to the wire from the electrode vessel and readings are taken at intervals of 1 minute, 2 minutes, and 3 minutes after the first mixing of the serum and the quinhydrone-salt solution. Slight drifts may be observed, and if they amount to more than 0.2 mv. in 3 minutes and are consistently in one direction, the dilution must be altered. If the drift is toward more positive potentials, the amount of quinhydrone-salt solution must be decreased or the amount of serum increased, and vice versa when the drift is toward more negative potentials. Duplicate determinations--that is, readings that do not vary by more than 0.2 mv.--must be made at the dilution which seems to eliminate the drift. Usually, the E.M.F. shows a slight maximum--rarely, a minimum--at the second minute reading, but sometimes there is a consistent drift of 0.1 or 0.2 mv. in 3 minutes. When the drift has been reduced to 0.1 or 0.2 mv. in 3 minutes, the first minute value is taken as the best E.M.F. if the drift is consistent, or the second minute

value if there is a maximum or minimum. After each determination the electrode vessel is thoroughly rinsed with distilled water, by separating the tubes and playing a stream through the outer tube and over the surface of the inner tube. Occasionally, however, it may require more vigorous methods of cleaning.

6. Calculation of pH

The pH of a sample of serum is calculated from the pH of the phosphate standard and the potentials of the serum and standard by substituting these values in the general equation:

$$\text{pH}(\text{unknown}) = \text{pH}(\text{known}) + \frac{\text{E.M.F.}(\text{known}) - \text{E.M.F.}(\text{Unknown})}{0.0001983 T}$$

where T is the absolute temperature of the unknown at the time of the determination. The pH of the phosphate standard--used as the "known" pH in calculating the pH of the serum--is itself similarly calculated from the observed potentials of the phosphate and N/10 HCl standards and the assumed pH of 1.08 for the N/10 HCl.

Since the E.M.F. values observed are functions of the temperature, they must be compared at the same temperature. It was our practice to correct other E.M.F.'s to the temperature of the phosphate standard, by applying the temperature coefficients of the quinhydrone and saturated calomel half-cells, and to avoid temperature corrections greater than 0.2° or 0.3°C., equivalent to about 0.6 mv. The temperature coefficient of the calomel half-cell was considered always to be $\frac{d\text{E.M.F.}}{dt} = +0.8$ mv., but the sum of the two temperature coefficients of the quinhydrone half-cell, $\frac{d(0.0001983 T) \text{ volts} \times \text{pH}}{dt} = -0.2 \text{ mv.} \times \text{pH}$

and $\frac{dE.M.F.}{dt} = -0.7 \text{ mv.}$, varied with the pH. The temperature coefficients of the two half-cells are opposite in sign and tend to cancel each other, except, as very rarely happens, when the temperatures vary in opposite directions.

The calculation of the pH of a sample of serum involves three steps which are most easily explained by means of an example. Given the data:

Solution	t(cal.)	t(bath)	E.M.F.
N/10 HCl	23.2°	23.3°	389.7
Phosphate	23.3°	23.4°	30.2
serum	23.4°	23.5°	10.9

calculate the pH of the serum.

The first step is to calculate the pH of the phosphate standard. Applying the corrections mentioned in a previous paragraph, and correcting the E.M.F. of the acid to the temperature of the phosphate, we get $0.08 - 0.02 - 0.07$ or no significant correction to the nearest tenth of a millivolt. Next, we substitute in the general equation and get

$$pH = 1.08 + \frac{389.7 - 30.2}{58.78} = 7.196$$

The next step is to calculate the pH of the serum at the temperature at which it was determined. Applying the corrections as before, we get $-0.08 + 0.14 + 0.07$, or 0.1 to the nearest tenth of a millivolt. This, added to the observed E.M.F. makes a corrected value of 11.0. Substituting in the equation, we get

$$pH = 7.196 + \frac{30.2 - 11.0}{58.8} = 7.523.$$

The above is the pH at 23.5°C., and the last step is to correct this value to 38°C. by applying an empirical tempera-

ture coefficient. The temperature coefficient, which will be discussed in the next section, is in this case, $\frac{dpH}{dt} = -0.012$,

and applying it we get

$$7.523 - 14.5 \times 0.012 = 7.349.$$

RESULTS

A. General Plan of the Experiments

The general plan of the experiments was to study a wide variety of quinhydrone-dilution procedures and to compare them either directly or indirectly with the gasometric method. The quinhydrone-dilution procedures included the effective dilution of serum with a large number of solutions varying in the kind and concentration of salt and the concentration of quinhydrone. The salts most extensively investigated were the simple salts regularly found in serum, but tentative studies were also made on others, especially those of ammonium. Solutions containing various concentrations of the salts and various concentrations of quinhydrone were compared and their usefulness judged on the basis of the following criteria: (1) ease of eliminating the drift; (2) stability of the solution; (3) reproducibility of determinations at the effective dilution; and (4) ability to match the pH values determined by the gasometric method. The quinhydrone procedure described in the last section was selected because it best met all these requirements.

B. Effects of Variations in the Concentration
of Quinhydrone

Of the two constituents of the quinhydrone-salt solution, the quinhydrone is chiefly responsible for the elimination of the drift, and the relative volume of the quinhydrone-salt solution needed for the effective dilution of serum depends on the concentration of quinhydrone in it. The quinhydrone-salt solutions used in our earliest experiments were saturated with quinhydrone, but they were unsatisfactory in that they required too little quinhydrone solution for the effective dilution of the serum and because they were not uniform in concentration. The first is apparently the more serious fault because the electrode vessels used do not permit the precise measurement of as little as 0.10 cc. of quinhydrone-salt solution nor the thorough mixing of the quinhydrone-salt solution with the serum when the total volume is greater than 0.5 or 0.6 cc. Table I shows the effect on the ratio of volumes necessary to eliminate the drift of saturating various solutions with quinhydrone.

TABLE I

Salt	Concentration (in mols per liter)	Effective dilution (in cc. x 10 ⁻²)	
		Quinhydrone solution	serum
NaCl	0.154	10	23
NaCl	1.34	15	25
NaCl	2.56	15	23
NaCl	saturated	15	15
KCl	0.154	10	35
KCl	1.34	10	33
KCl	2.56	10	32
KCl	saturated	10	22
NH ₄ Cl	0.9%	upward drift	
NH ₄ Cl	3.9%	15	15
NH ₄ Cl	10.0%	downward drift	

The differences in the effective dilution of solutions saturated with quinhydrone depend first on the salt and to a less extent on its concentration, and they probably reflect the solubility of the quinhydrone in the solutions. A complete tabulation of all the salt solutions used would not be feasible and it would serve no good purpose; for the differences in the effective dilution with other salt solutions saturated with quinhydrone are similar to those with the solutions of NaCl and KCl. An exception must be made in the case of the ammonium salts, however, as is shown in Table I. A 0.9% solution or a 10% solution of an ammonium salt saturated with quinhydrone causes a drift that cannot be eliminated with any dilution feasible for the electrode vessels described, but there is a solution having an intermediate, definite concentration of the salt which, if saturated with quinhydrone, will eliminate the drift when the serum is diluted with an equal volume of quinhydrone-salt solution.

C. Comparison of Salts at One Concentration of Quinhydrone

The comparison of different salts on the basis of the criteria mentioned can best be made at one concentration of quinhydrone; for the effective dilution with all the solutions studied, except those of the ammonium salts, is the same when the concentration of quinhydrone is the same. The concentration of quinhydrone used in the comparison shown in Table II was 125 mgms. of quinhydrone per 100 cc. of solution, and the effective dilution was 0.25 cc. of quinhydrone-salt solution to 0.15 cc.

of serum. The only exception was the ammonium salt solution, which was saturated with quinhydrone and the effective dilution here was equal volumes of solution and serum. The table is shortened by showing only the ions most thoroughly investigated and by showing only one example each of the ammonium, magnesium, sulphate and nitrate ions. The Ringer's solution was the conventional warm-blooded solution containing Na, K, Ca, and Mg.

TABLE II

Salt	Concentration (in mols per liter)	Best E.M.F. (in mv.) (3 determina- tions)	Variation (best E.M.F.)
NaCl	0.154	10.3	10.2 to 10.4
NaCl	1.34	16.1	15.8 to 16.1
NaCl	saturated	- 2.9	- 2.5 to -3.1
KCl	0.154	9.5	9.3 to 9.5
KCl	1.34	10.3	10.2 to 10.3
KCl	saturated	0.7	0.3 to 0.9
NH ₄ Cl	3.9%	33.9	33.9 to 36.1
MgCl ₂	1.34	38.2	37.6 to 38.7
Na ₂ SO ₄	1.34	23.9	23.2 to 24.1
NaNO ₃	1.34	9.4	9.3 to 9.8
Ringer's	-	9.8	9.7 to 10.2

All of the solutions studied, except the ammonium, are apparently stable, and all of them will eliminate the drift. The ammonium solutions soon become purple in color and each determination gives an apparent pH more acid than the last; but the KCl and NaCl solutions gave identical E.M.F. values, respectively, when the serum was effectively diluted with a fresh solution and with a solution that had stood exposed to air for 5 hours at 20° to 25°C. The other salt solutions were not so

thoroughly investigated, but they were stable for at least an hour or so. It will be noted that the 1.34 molar or 10% KCl solution best meets the important criterion of reproducibility, shown in Table II under the "variation in best E.M.F." and this implies that it also best meets the criterion of ability to match the gasometric method; for the correction necessary to make the pH numbers, determined by any quinhydrone procedure using the conventional corrections, match those obtained by a standard method can be determined if the quinhydrone procedure gives definite E.M.F. values.

D. Comparison of Different Concentrations of
Quinhydrone and Different Concentrations
of KCl

The ratio of volumes of a quinhydrone-salt solution and serum needed to eliminate the drift is a function solely of the concentration of quinhydrone in the quinhydrone-salt solution, but the apparent pH at a given concentration of quinhydrone varies with the concentration of the salt. It decreases as the concentration of the salt increases until it reaches a certain minimum and then increases as the concentration of salt increases. Thus in Table III it is seen that the pH for 100 mgms. quinhydrone varies from 7.355 to 7.330, 7.367, and 7.628, as the concentration of the KCl varies from 0.154 M to 1.34 M, 2.00M, and saturated, respectively. Further experiments on this point have shown that the apparent pH at one concentration of quinhydrone, decreases as the concentration of KCl increases to about 1.3M, remains practically stationary between concentrations of 1.3M and 1.9M,

and increases as the concentration of KCl increases above 1.9M. Table III also shows that if the quinhydrone concentration of any one KCl solution be increased, the apparent pH decreases although the concentration of salt in the final mixture of salt solution and serum also decreases. Table III is shortened to show only a few different concentrations of KCl and quinhydrone, but the relations just mentioned are apparent in the table. The successive variations in the apparent pH within any one group of Table III are near the limit of experimental error, but the total variation within a group or the variation from group to group is well outside the limit.

TABLE III

Concentration KCl (Mols/liter)	Concentration Quinhydrone (mgms/100cc.)	Effective dilution (cc. x 10 ⁻²)		Apparent pH
		Quinhydrone solution	serum	
0.154	100	25	13	7.355
.154	200	20	19	7.332
.154	saturated	10	35	7.325
1.34	50	30	12	7.339
1.34	100	25	12	7.330
1.34	125	25	14	7.326
1.34	150	20	15	7.319
1.34	200	20	20	7.306
1.34	250	15	29	7.297
1.34	saturated	10	32	7.293
2.00	100	25	12	7.367
2.00	200	20	20	7.329
2.00	saturated	10	32	7.326
saturated	100	25	13	7.628
saturated	200	20	20	7.487
saturated	saturated	15	29	7.434

The gasometric pH of this sample of serum was 7.328.

The tables so far presented show the reasons for selecting the

quinhydrone procedure described. 10% (1.34M) KCl was chosen for the salt solution because it was found to give the best reproducibility, but the concentration need not be controlled closer than to 10% of the total. 125 mgms. of quinhydrone per 100 cc. of salt solution was chosen as the best concentration of quinhydrone because it gives easily measured volumes of salt solution and serum at the effective dilution and because the apparent pH matches the gasometric pH satisfactorily. The quinhydrone concentration need not be controlled closer than 5%.

E. Comparison of pH Values Determined by
the Chosen Quinhydrone Procedure
and by the Gasometric Method

The pH values were determined by the quinhydrone procedure at 20° to 25° and were corrected to match the gasometric values at 38° by means of empirical corrections. The correction or temperature coefficient, $\frac{dpH}{dt} = -0.012$, gives satisfactory agreement when the gasometric pH values range from 7.3 to 7.5, but the corrected quinhydrone values are more acid than the gasometric values when the latter are less than 7.3, and more alkaline when the gasometric values are greater than 7.5. The difference between the quinhydrone pH at t (20° to 25°C.) and the gasometric pH at 38°C. increases as the pH increases, but the rate of increase is not regular. It was found, however, that the corrected quinhydrone values mentioned above could be made to check quite closely with the gasometric pH values between 7.0 and 7.6 by means of an additional correction to be added to corrected pH values less than 7.4 and subtracted from those greater than 7.4. This second correction is $\frac{\Delta^2}{3.6}$,

where A is the difference between the first corrected value and
7.4. Table IV gives some examples of its application.

TABLE IV

Quinhydrone pH Values					Gasometric pH
pH at t	pH at 38° (pH at t - .012(38-t))	A	$\frac{A^2}{3.6}$	corrected pH	
7.028	6.843	.557	.086	6.929	6.935
7.344	7.168	.232	.015	7.183	7.191
7.549	7.363	.037	.000	7.363	7.360
7.872	7.694	.294	-.024	7.670	7.656

Table V shows how the difference between the quinhydrone
pH values at t and the gasometric values at 38°C. increases with
the pH and shows the average temperature coefficients which will
make them match at various pH ranges.

TABLE V

Group of Expts.	pH range (pH at t 20-25°C.)	Average difference (between pH at t and gasometric pH at 38°C.)	$\frac{dpH}{dt}$
A	about 7.00	0.090	0.006
B	7.15 to 7.25	0.145	0.0095
C	7.25 to 7.35	0.154	0.0099
D	7.35 to 7.45	0.178	0.0116
E	7.45 to 7.55	0.183	0.0119
F	7.55 to 7.65	0.187	0.012
G	7.75 to 7.85	0.212	0.0139
H	7.90 to 8.00	0.354	0.0234

Table VI shows the maximum and average deviation of the
quinhydrone from the gasometric values when the former pH values
are determined by use of the correction $\Delta pH = -0.012\Delta t$, and
by the corrections illustrated in Tables IV and V respectively.
The same groups of experiments and pH ranges are shown in Tables
V and VI.

TABLE VI

Group of Expts.	$\Delta \text{pH} = -0.012 \Delta t$		$7.4 \pm A^2/3.6$		$\frac{dpH}{dt}$ of Table V.	
	average	maximum	average	maximum	average	maximum
A	+0.094	+0.096	0.003	0.006	0.002	0.004
B	+ .037	+ .047	.009	.018	.008	.016
C	+ .031	+ .040	.016	.020	.009	.016
D	+ .008	+ .018	.006	.013	.008	.017
E	$\pm$.004	$\pm$.008	.003	.008	.004	.010
F	$\pm$.004	$\pm$.009	.004	.008	.004	.009
G	- .028	- .038	.019	.024	.008	.010
H	- .173	- .181	.131	.140	.009	.011

DISCUSSION OF RESULTS

The tables of the preceding section summarize the results of exploring a wide variety of quinhydrone procedures and of comparing the pH values determined by the chosen quinhydrone procedure with those determined by the gasometric method. Tables I, II and III summarize the exploratory experiments, and IV, V, and VI the comparison of method studies. These tables have been commented on individually, but it may be interesting to discuss them further as groups.

The exploratory studies summarized in Tables I, II, and III were necessary for the development of an accurate quinhydrone-dilution method, but they throw little light on the peculiarities of the quinhydrone-serum half-cell. They show that elimination of the drift depends on the concentration of quinhydrone, but they don't show why there is a drift; why a certain concentration of quinhydrone eliminates it; or why the direction of the drift changes when the concentration of quinhydrone diminishes or increases beyond the optimum concentration. In the same way, the

exploratory studies show that the E.M.F. observed when the drift is eliminated is a function of the kind and concentration of salt used in the quinhydrone-salt solution, but they throw no light on the mechanism by which the salt operates.

It may be that the known conditions that interfere with the quinhydrone determination of the pH of serum, namely the slight alkalinity and the presence of salts and proteins, can account for all the peculiarities of the quinhydrone-serum half-cell. If so, the mechanism would probably involve the salt and protein errors, shown by Linderstrøm-Lang and others to be inherent in the quinhydrone method, or some such interaction of the constituents of the half-cell (10,11). A satisfactory answer to the question raised by the exploratory studies might be found by extending the studies farther, but the evidence of the studies reported here warrants only the selection of a certain quinhydrone-dilution procedure.

Tables IV, V, and VI show the comparison of the quinhydrone-dilution and gasometric methods in so far as agreement of pH values is concerned, but they say nothing about the relative advantages and disadvantages of the quinhydrone-dilution method. The chief advantages of the quinhydrone-dilution method over the gasometric method are the simplicity of the set-up, the ease and speed with which the determinations can be made, and the fact that the pH values of both equilibrated and non-equilibrated samples can be readily determined. The chief disadvantage of the quinhydrone-dilution method is that it has not yet been made applicable to blood, or to plasma or serum containing hemolyzed blood.

The relative speed of the quinhydrone determinations can be judged by comparing the time required for a determination by

each method. A quinhydrone determination requires on the average about seven minutes if the potentials are read 1 minute, 2 minutes, and 3 minutes after the first mixing of the quinhydrone-salt solution with the serum. Each part of the gasometric determination--the determination of the serum CO_2 content and of the percent CO_2 in the gas mixture, requires from 20 minutes to half an hour, so that from 6 to 8 quinhydrone determinations can be made in the time required for one gasometric determination.

Perhaps the chief advantage of the quinhydrone method is that the pH of a sample of serum can be determined without regard to the gas tension with which it was in equilibrium. Little has been said about the non-equilibrated samples of serum, that were prepared and analyzed by the quinhydrone method in connection with most of the collaborative experiments, because those pH values were not checked by the gasometric method. We had no reason to believe, however, that they were any less accurate than those of the equilibrated samples analyzed along with them. The pH values of duplicate samples agreed well, they reflected the changes produced by adding known quantities of acid or base to certain control tubes, and they gave consistent results when used with other data in the calculations of the collaborative experiments.

The quinhydrone-dilution method is not applicable to blood nor to plasma or serum containing hemolyzed blood, and the prospects of developing a practicable method are not bright. The drift can be eliminated in any case, but the reproducibility is poor, especially with blood, where it is of the order of 1 to 3 millivolts, equivalent to 0.02 to 0.05 pH. The apparent pH numbers are too alkaline. With plasma or serum, the reproducibility is satisfactory except when there is extensive hemolysis,

but the apparent pH numbers are too acid. The poor reproducibility is probably due to the fact that, with blood or with plasma or serum containing much hemolyzed blood, the volume that can be effectively diluted by a volume of salt solution, saturated with quinhydrone, feasible for the present electrode vessels, is too small to be measured with sufficient accuracy to give the reproducibility desired. This condition might be overcome by another type of electrode vessel, but that alone would not solve the problem. It seems probable that the correction needed to change the apparent pH at 20° to 25°C. to match the standard pH varies with the oxygen capacity and oxygen content of blood and with the extent of the hemolysis in the case of serum or plasma. If this is so, these variables would have to be known for every sample before a certain correction could be applied, granting that a correlation had been made between the corrections and the variables. The quinhydrone-dilution method here described was developed with serum showing little if any hemolysis, and we learned by experience to judge by the appearance of a sample whether or not hemolysis had been too extensive for the sample to be accurately analyzed by the quinhydrone-dilution method.

SUMMARY

A quinhydrone-dilution method for the pH of blood serum has been described. In this method the drift in potential, an undesirable feature of the conventional quinhydrone-serum half-cell, is eliminated by mixing measured volumes of serum and a solution containing 10 grams of KCl and 125 milligrams of quin-

hydrone per 100 cc. of solution, in a graduated capillary electrode vessel. The apparatus used and the procedure followed have been described in detail, and a sample calculation of pH has been given.

Tables have been presented which summarize the comparison of a large number of quinhydrone procedures, and which compare the pH values determined by the chosen procedure and the gasometric method. The pH values determined by the quinhydrone-dilution method check those of the gasometric method within the experimental error of the latter.

The relative advantages and disadvantages of the quinhydrone-dilution method over the gasometric method have been discussed. The chief advantages of the quinhydrone-dilution method are its speed and the fact that it is applicable equally well whether or not the CO_2 tension of the serum is known. Its chief disadvantage is that it is not applicable to blood nor to plasma or serum containing much hemolyzed blood.

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